SURVIVAL AND DEVELOPMENT OF PINK SALMON (Oncorhynchus gorbuscha) EMBRYOS AND FRY AS RELATED TO EGG SIZE AND QUANTITATIVE GENETIC VARIATION

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ABSTRACT

The effect of egg weight on survival and development of pink salmon (*Oncorhynchus gorbuscha*) embryos, alevins, and fry was analyzed; in addition, embryo survival was investigated in relation to additive genetic variation. Embryonic survival to eyeing, development time to hatch, yolk weight, somatic tissue weight, yolk use rate, somatic tissue growth rate, and the survival of first-feeding fry was recorded relative to egg weight. The analyses demonstrated significant egg weight effects on development time to hatch, yolk weight, somatic tissue weight, yolk use rate, and somatic tissue growth rate of alevins. Weight and length of post-emergent fry (17 weeks post-ponding) were also significantly affected by initial egg weight. However, egg weight did not affect survival of eyed eggs or fry. Differential family-specific survival of eyed eggs indicated the presence of significant additive genetic variation.

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CHAPTER I.

EGG SIZE EFFECTS ON SURVIVAL AND EARLY DEVELOPMENT OF PINK SALMON EMBRYOS AND FRY

INTRODUCTION

From an evolutionary perspective, the primary function of gametes is not to carry energy but to transmit information; and because information can be transferred in very small packages, eggs can be very small and numerous. However, the optimum egg size of a particular female should be the size which yields the maximum number of surviving progeny (Calow 1981). This suggests a trade-off between the number and size of eggs. Pacific salmon (*Oncorhynchus* spp.) investment in egg production is dependent on many factors including ocean feeding conditions and migration timing (Grachev 1971). Fecundity and egg size are at odds in salmon egg production.

Salmon fecundity varies among populations within species (Healey and Heard 1984; Fleming and Gross 1990), among females within populations (McGregor 1922), and among years within a population (Beacham 1982). Egg size also varies among populations (Beacham and Murray 1987) and among females within populations (Beacham and Murray 1985). Fecundity and egg size are important life history characteristics that are closely linked through an ecological trade-off, i.e., the number of eggs produced is limited by the size of the eggs. Therefore, average egg size increases at the expense of fecundity and vice versa.

Salmon egg production is affected by many factors including spawning time (Beacham and Murray 1987), latitude of natal stream (Fleming and Gross 1990), and distance of freshwater migration (Beacham and Murray 1993). Perhaps the most important determinant of egg size and fecundity is female size. Therefore, changes in average body size of mature salmon may have dramatic impacts on egg production.

Average size in many salmon populations declined from the early 1970's to the 1990's (Kaeriyama 1998; Marshall and Quinn 1988). Helle and Hoffman (1995) reported a 46% reduction of mean male carcass weight for two stocks of chum salmon (*O. keta*) over a 20-year period 1972-1992. Similar size decreases have also been reported for all species of Pacific salmon: pink (*O. gorbuscha*, Ricker et al. 1978; Ricker 1980, 1981; Marshall and Quinn 1988); coho (*O. kisutch;* Marshall & Quinn 1988; Ricker & Wickett 1980); chinook (*O. tshawytscha*; Ricker 1980; Fagen 1988), and sockeye (*O. nerka*; Nelson et al. 1986). At Auke Creek in Juneau, the average length of late-run female pink salmon has declined by about 5 cm since the early 1970's (personal communication,

S.G. Taylor, US NMFS Auke Bay Laboratory, Juneau, AK). Hypotheses for explaining these size reductions include size selective fishing, climatic changes, and density-dependence. Regardless of the cause, decreases in the size of mature salmon could significantly impact survival, recruitment, stock dynamics, and as a consequence harvest management.

Reductions in body size at maturity may affect subsequent generations in several ways. First, fecundity of female salmonids is positively correlated to body size (Foerster and Pritchard 1941, Fowler 1972; Bromage et al. 1990). Therefore, smaller females produce fewer eggs. In addition, smaller females produce smaller eggs (Fowler 1972; Kazakov 1981; Beacham and Murray 1985; Bromage et al. 1990; Fleming and Gross 1990). In hatchery broodstocks, the relationship between female size and egg size is evident in the interannual variation of average body and egg size. Smaller eggs also produce smaller fry (Yastrebkov 1966; Smirnov 1975; Koski 1975; Kazakov, 1981; Beacham and Murray 1990; From and Rasmussen 1991) which have slower growth (Gall 1974; Pitman 1979) and may have reduced survival due to their diminished ability to escape predators (Bams 1967; Mead and Woodall 1968; Parker 1971; Healey 1982). Smaller eggs may also suffer greater mortality during development, particularly during emergence and first feeding. This reduced survival may be a consequence of reduced endogenous nutrition (yolk) stores (Gall 1974; Kincaid et al. 1977).

Data from the AFK Hatchery of Prince William Sound Aquaculture Corporation (PWSAC; Cordova, Alaska) suggest that mortality occurring during development from embryo to first-feeding fry may be correlated with egg size in pink salmon. In 1991 and 1993, cumulative mortality from embryo to newly emerged first-feeding fry exceeded 15% despite the fact that normal mortality at AFK is usually negligible (personal communication, Dr. T. Linley, PWSAC, Cordova). Coincidentally, in 1991 and 1993 average egg size and average body size of the broodstock were especially small. Mortality due to small size of embryos would be difficult to detect in wild stocks because obtaining accurate information about embryos and fry is not feasible. Nevertheless, fry from wild stocks may have increased mortality in years when egg size is small. Therefore, the decline in body size of salmon may result in lower productivity not only because of lower fecundity but also because of increased fry mortality due to decreased egg size.

Decreased body size of female salmon is correlated with decreased egg size and survival of fry due to the diminished ability of small fry to forage, assimilate food, and avoid predators (Beacham and Murray 1985; Parker 1971). Another nutritional effect on survival at emergence and first feeding has also been proposed because the fry from small eggs have an insufficient supply of endogenous nutrition; they reach Maximum Alevin Wet Weight (MAWW; Bams 1970) earlier and require exogenous sources of nutrition earlier. The need for food forces fry to emerge prematurely from the gravel and this early emergence has been repeatedly correlated with poor marine survival in wild stocks (Taylor 1980; and personal communication S.G. Taylor).

In order to determine the effect that egg size has on fry survival and investigate the causes of any increased mortality, two hypotheses were tested. The first hypothesis was that small eggs lack adequate supplies of raw materials, such as maternal RNA and endogenous nutrition, so they cannot support normal development and the embryos die before beginning a normal, free-living existence. The second hypothesis asserts a nutritional problem. Small eggs contain insufficient stores of endogenous nutrition. Consequently, embryos and fry exhaust nutritional stores faster, which causes fry to emerge and require exogenous nutrition prematurely. That is to say, small-egg fry reach MAWW sooner. This has implications for salmon ecology and population dynamics as well as fish cultural implications: early emergence in nature is correlated with poor survival.

The developmental hypothesis (H1) was investigated by observing mortality (to eyeing) for a range of egg sizes. If H1 were true, increased mortality during embryonic development of small-egg embryos would be observed. The nutritional hypothesis (H2) was investigated by observing development time to hatching, rate of yolk use after hatch, and growth rate of embryos within a range of egg sizes. If H2 were true, shorter development times, lesser yolk reserves and smaller body sizes in alevins and fry from small eggs would be observed.

MATERIALS AND METHODS

Overview – The effect of egg size on various aspects of early pink salmon development was studied. A preliminary random sample of female pink salmon determined the range of egg diameters that could be expected to be observed during an eight-hour sampling period. Based on the random sample, target egg diameter categories were established and a second sample, stratified with respect to each female's average egg diameter, was taken. Eggs obtained were fertilized and incubated in randomized compartments and emergent fry were observed in growout containers. Observations of incubating eggs and fry were statistically analyzed by linear regression.

Gamete Acquisition and Culture

Study Population

Pink salmon gametes were obtained from the Gastineau Hatchery in Juneau, Alaska. Douglas Island Pink and Chum (DIPAC) operates Gastineau Hatchery and obtained their pink salmon broodstock from nearby Salmon Creek. Fertilization and incubation occurred at Kowee Creek Hatchery on Douglas Island about 3 km from the Gastineau Hatchery and Salmon Creek.

Preliminary Random Sample

On 29 August 1996 a random sample of 41 female pink salmon was taken. Ten eggs from each female were extruded onto a specially designed ruler. The ruler cradled eggs in a single-file row so that the average egg diameter of each female could be recorded (Figure 1). Based on the random sample, 15 egg diameter categories, that ranged from 5.1 to 6.5 mm at 0.1 mm intervals, were established.

Stratified Sample

On 30 August 1996 female pink salmon were sampled with respect to egg diameter. Ten eggs were extruded from approximately 300 live females and the average egg diameter of each female was determined. Due to time constraints, the stratified

sample and fertilization did not occur on the same day. Therefore, females that possessed an egg diameter of interest were marked with a numbered floy tag and placed in a holding tank until the following day. Each egg diameter category was represented by at least 4 females, except 5.1, 5.2, and 6.5 mm (Figure 1). The smallest (5.1 and 5.2 mm) and largest (6.5 mm) egg diameter categories were difficult to fill because of their rarity in the population. Therefore, additional females with egg diameters of 5.3 and 5.4 mm were collected to complete the design.

Mating Design and Incubation

On 31 August 1996, gametes were collected and fertilization was completed. Sperm from 30 spermiating males, selected at random, and eggs from 60 fully ovulating females, with known egg diameters, were obtained. Sperm, collected by expression, and eggs, obtained by excision, were placed in dry labeled containers. Sperm motility was verified by microscopic observation. Gametes were transported on ice to Kowee Creek Hatchery.

A nested hierarchical mating design was employed to create 60 half-sib families. Sperm from a single, randomly chosen male fertilized eggs from two females randomly chosen from among all females. Each full-sib family (single male mated to single female) was split into two replicates and randomly placed into 120 compartments of FAL Heath™ incubator trays. Fertilization was by the dry method and fertilized eggs were rinsed before being placed into incubator compartments. The fertilized eggs were incubated in free-flowing, ambient Kowee Creek water.

Fry Growout

From 9-11 April 1997, sub-samples of pre-emergent fry were transported to thirty 60-liter growout tanks at DIPAC. Due to the failure of one half-sib family, presumably due to an overripe female, only 59 of the original 60 half-sib families remained. Fifty fry, from each of 29 half-sib families, were adipose fin clipped and randomly placed as a group in one of 29 tanks. Fifty fry with adipose fins intact, from each of 29 of the remaining 30 half-sib families, were randomly placed as a group in one of the 29 tanks that already contained clipped fry. Therefore, 29 tanks contained 50 clipped and 50

unclipped fry from each of two half-sib families. The remaining half-sib family was placed in the remaining tank but 100, instead of fifty, fry were added in order to keep stocking density constant among all tanks. Fry were fed commercial starter diet ad libitum and were raised under ambient photoperiod.

Observations

Females and Eggs

Parental mid-eye to fork-tail (MEFT) lengths and post-spawning weights were recorded. Entire egg masses were weighed and sub-samples of egg masses were counted and weighed to determine fecundity. Average egg weights for individual females were determined by weighing about 30 blotted eggs per female.

Embryos and Alevins

After fertilization, eggs and alevins were sampled eight times (Figure 2). Six sampling dates were selected for laboratory examination. The dates selected were 8/31/96 (16 hours post fertilization, Fertilization sample), 1/10/97 (Hatch), 2/24/97 (Post-Hatch 1), 3/28/97 (Post-Hatch 2), 4/16/97 (Post-Hatch 3), and 5/18/97 (Emergence). At each sampling, 10 individuals from each full-sib family (5 from each replicate) were preserved in a 10% formalin solution.

An initial pick-off of "blank" eggs was performed on 1 September 1996. On 18 October 1996, eyed eggs were physically shocked and mortalities were counted and removed. The date at which 50% of a compartment had hatched was recorded. Ambient temperatures and mortalities were recorded so that accumulated temperature units (ATU) and survival rates could be determined.

Fry

Daily temperature and mortalities were recorded. On 8 August 1997, all remaining fish were euthanized in MS-222 and preserved in a 10% formalin solution for laboratory examination. It should be noted that throughout this thesis, a distinction is

made between the terms “alevin” and “fry”. “Alevin” refers to observations of salmon in incubators, while “fry” refers to observations of salmon after ponding.

Laboratory Protocol

Fertilization Sample -- Failure of fertilization would result in the overestimation of mortality in embryos. The fertilization sample was used to verify the viability of the crosses. Samples were removed from formalin solution, rinsed with fresh water, then fixed in 10% acetic acid for 2 minutes. Samples were then placed in alcohol and examined under a dissecting microscope for cell cleavage. Cleavage was observed in more than 90% of the samples; all crosses were retained in the experiment.

Alevin Samples -- Alevin length, somatic tissue weight, yolk weight, and yolk area measurements were observed on alevin samples taken at Hatch, Post-Hatch 1, Post-Hatch 2, Post-Hatch 3, and Emergence. Alevins were removed from vials, blotted dry and placed under a digital camera. An image file was created and alevins were measured for length and yolk area using Optimas™ image analysis software. This software allows the user to draw lines and infinitely sided polygons on top of images and calculate length and area. Linear and areal measurements of images were calibrated to scale and data was exported to an Excel™ spreadsheet.

Length was measured to the nearest 0.1 mm by drawing a line from mid-eye to the caudal peduncle. Yolk area was calculated to the nearest mm^2 by drawing a multi-sided polygon around the yolk mass. Alevins were weighed intact then yolk mass was dissected away and yolk was weighed separately. Somatic tissue weight was calculated by subtracting yolk weight from total weight.

Fry Samples -- Preserved fry were blotted dry with paper towels and weighed. Length was measured from mid-eye to the caudal peduncle with the Optimas™ image analysis system. All weights were measured to the nearest milligram with an electronic balance.

Data Analysis

Fecundity was estimated by multiplying egg mass total weight by the number of eggs per unit of weight in the weighed subsample. Embryo survival was calculated for the interval from initial pick-off to eyeing. In order to stabilize variance, embryo survival data was transformed to the empirical logit (Agresti 1990).

$$\text{logit (survival)} = \log [(y_i + ½)/(n_i - y_i + ½)]$$

where y_i is the number of mortalities and n_i is the initial number alive of the i^{th} replicate incubator. Fry survival was calculated from ponding to 7 weeks post-ponding and for the complete duration of the fry experiment (ponding to 17 weeks post-ponding). Fry survival data was transformed with the arcsine transformation (Sokal and Rohlf 1981).

$$Y' = \text{arcsine } \sqrt{(x_2 / x_1)}$$

where Y' is transformed survival, x_1 is the initial number alive and x_2 is the final number alive. Development time to hatch was calculated by determining the date at which 50% of an incubator compartment had hatched and computing the number of days since fertilization. Development time to hatch data was transformed by the square root function.

Yolk area, yolk weight, alevin length, fry length, and fry weight represent individual fish records but somatic tissue weight represents replicate incubator cell averages. Somatic tissue weight is the average weight of 5 alevins from an incubator compartment with yolk mass intact, minus the average weight of those alevins' yolk masses (averaging was necessary because individual fish were not followed through sampling). Yolk use and weight growth rates also represent incubator cell averages and were calculated as instantaneous rates.

$$Y' = ((\ln w_2 - \ln w_1)/ t_2 - t_1) \times 100$$

where Y' is instantaneous rate (%/day) and w_1 and w_2 are weights on days t_1 and t_2. Weight growth rate, for the interval from Hatch to Emergence, and yolk use rate during all intervals were transformed by the natural log function to stabilize variance. Fry weight was transformed with the square root function.

Observations of embryos, alevins and fry were analyzed with regression analysis.

$$\hat{Y} = a + b X$$

where $\hat{Y}$ is the response variable; X is the independent variable egg weight; a is the Y-intercept and b is the slope of the regression line.

Variables and regression residuals were examined for normality and outliers. Stem and leaf and box plots helped determine appropriate data transformations. Grubbs' (1969) test was used to identify outliers. Some datasets contained multiple outliers but most were due to a common developmental disorder where alevins accumulate fluid in the yolk sac. This condition produces individuals that appear bloated, are abnormally stunted and is usually not fatal until swim-up. Data transformation and outlier treatment is addressed in Appendix I.

Local regression analyses of fecundity and egg size on female length was completed with a Loess function employing a span of 0.75. All statistical analyses were performed with the statistical program S-Plus 4.5 Professional Release 1 (MathSoft, Inc. 1998).

RESULTS

Females and Eggs

Due to the large number of females sampled and the nature of the stratified sampling environment, i.e., wet and windy, it was not feasible to obtain egg weight measurements from live females. However, egg diameter measurements were rapid and an effective surrogate measurement of egg weight. Average egg diameter, calculated during the stratified sample, was highly correlated (r=0.910) with average egg weight which was calculated under laboratory conditions (Figure 3). Egg weights spanned a considerable range with the smallest average egg weight (0.105 g) being 55% of the largest average egg weight (0.192 g).

Female body size had a marked effect on both average egg weight and fecundity. Smaller females tended to produce smaller eggs although there appeared to be a flatter relationship among eggs from the smallest females (Figure 4). In addition, smaller females were associated with lower fecundities (Figure 4).

Embryos and Alevins

Survival

Overall, embryo survival to eyeing was nearly 90%. Regression of the logit of survival against egg weight was not significant (P=0.416) (Figure 5). One female produced embryos with extremely low survival (10.1%), presumably due to overripe eggs. This female's progeny were removed from the analysis.

Development Time

The number of days to 50% hatch varied from 116 to 130. The average number of days to hatch was 122.2. Egg weight had a significant effect on development time to hatch (P<0.001). Generally, development time increased with increasing egg weight (Figure 6).

Yolk Weight

Egg weight had a highly significant effect on yolk weight at all sampling episodes (all $P<0.001$). Alevins derived from the largest eggs also had the largest amount of yolk (Figure 7). However, the slope of the regression decreased with each succeeding sample. In addition, the amount of variation accounted for by the regression (r^2) also decreased.

Yolk Area

Yolk area was highly correlated with yolk weight. Therefore, egg weight effects on yolk area were similar to egg weight effects on yolk weight. These results are reported in Appendix II.

Somatic Tissue Weight

Egg weight had a highly significant effect on somatic tissue weight at all sampling episodes (all $P<0.001$), with somatic tissue weight increasing with increasing egg weight. Unlike the yolk weight regressions, the tissue weight regressions became more steeply sloped and accounted for a larger percentage of variation at later sampling episodes (Figure 8). Somatic tissue weight at Emergence ranged from 0.182 to 0.306 grams.

Alevin Length

Egg weight had a significant effect on fish length at Hatch (all $P<0.001$). Alevins derived from heavier eggs tended to longer. Alevin length regressions became more heavily sloped and accounted for a larger percentage of variation at later sampling episodes (Figure 9). Alevin length at Emergence ranged from 2.56 to 3.08 cm.

Yolk Use Rate

Egg weight had a significant effect on yolk use rate during all intervals (all $P<0.05$) with the exception of Post-Hatch 3 to Emergence ($P=0.101$). The general trend was for alevins derived from smaller eggs to have greater instantaneous yolk use rates (Figure 10).

Somatic Tissue Growth Rate

Egg weight had a significant effect on somatic growth during the interval from Post-Hatch 3 to Emergence ($P<0.001$), and the overall interval from Hatch to Emergence ($P<0.001$). During these intervals, alevins derived from larger eggs tended to have greater rates of instantaneous somatic growth (Figure 11). However, the relationship was not significant during the first three intervals.

Fry

Survival

At 7 weeks post-ponding, early fry survival was not significantly affected by egg weight ($P=0.839$) (Figure 12). Early fry survival was high and averaged 94%. Overall fry survival at the termination of the experiment, 17 weeks post-ponding, was low, 44%, and was also not significantly affected by egg weight ($P=0.139$). Survival results may have been complicated due to a period of massive mortality in early June that was most likely related to prolonged freshwater rearing (Figure 13). This poor survival may have masked egg weight effects.

Weight and Length

Egg weight had a significant effect on fry weight and fry length at the end of fry growout (both $P<0.001$). There was a general trend for increased fry weight and length among fish derived from larger eggs (Figures 14). Final fry weight averaged 1.35 g and ranged from 0.18 g to 4.37 g. Final fry length ranged between 2.60 cm and 6.65 cm, while average length was 4.46 cm.

DISCUSSION

Females and Eggs

Average egg weight and fecundity were both related to female body size. The positive relationship between egg weight and female size corresponds with similar findings among other salmonids (Fowler 1972; Kazakov 1981; Beacham and Murray 1985; Bromage et al. 1990; Fleming and Gross 1990). Likewise, the positive relationship between fecundity and female size was similar to that found by Foerster and Pritchard (1941), Fowler (1972), and Bromage et al. (1990). These relationships underscore the importance of the current downward trend in adult maturation size because decreases in average body size may have important implications for stock dynamics due to direct impacts from fecundity reductions and indirect impacts due to reduced egg sizes.

Perhaps, the existence of a biological obligatory minimum egg size mitigates egg size impacts. Evidence for such a minimum egg size may be demonstrated in Figure 4. Egg weight appears to level off at about 0.120 g while female length continues to decrease. A minimum egg size may ensure that developing embryos and fry have resources that are sufficient to complete development and incubation without abnormality or excessive mortality. A minimum egg size may also be environment dependent and would thus explain egg size variability among populations.

The downside of a minimum egg size is the trade-off between egg size and egg number. Females must allocate resources to produce eggs above the minimum size, therefore the possible number of eggs a female can produce is lessened. This may also be demonstrated in Figure 4, where it appears that as egg weight begins to level off at the minimum egg size, fecundity continues to decrease with respect to female length. During times when mature salmon body sizes are smaller, smaller females may reduce egg quantity in order to create viable eggs above the minimum size. This theory is in agreement with Fleming and Gross (1990) who reported that Pacific salmon egg size was stable between years but egg number and total egg production varied significantly. They postulated that atresia of maturing follicles is likely the mechanism for regulating the number of eggs so that eggs will be optimally sized for their environment.

Embryos and Alevins

Survival

This research found no evidence to support H1, that small eggs lack an adequate supply of raw materials to support normal embryonic development. Significant egg weight effects on survival to eye were not observed. Working with rainbow trout, Springate and Bromage (1985) found similar results suggesting that egg size may not be critical to early survival. This result is not surprising since at eyeing, yolk was still plentiful for embryonic development regardless of initial egg weight. However, other researchers have found positive and negative egg size effects on early survival. Fowler (1972), with chinook, and Pitman (1979), with rainbow trout, found that increased egg sizes had significant negative effects on pre-hatch survival. Conversely, Gall (1974) found a positive correlation between egg size and survival to hatch of rainbow trout embryos.

Egg weight effects on survival would likely occur later in development as yolk and other supplies are depleted and as exogenous feeding begins. Beacham et al. (1985) found that chum alevins from small eggs had lower survivals from hatch to yolk absorption. Jonasson (1993) also found a positive relationship between egg size and survival of Atlantic salmon during the period from eyeing to 12 weeks on exogenous food.

Development Time

Previous work by Kazakov (1981) with Atlantic salmon (*Salmo salar*), Wallace and Aasjord (1984) with char (*Salvelinus alpinus*), Beacham et al. (1985) with chum salmon, and From and Rasmussen (1991) with rainbow trout (*Oncorhynchus mykiss*) found no correlation between egg size and time to hatch. However, in this current research, egg weight had a significant effect on time to hatch. Time to hatch tended to increase with increasing egg size. Kristjánsson and Vøllestad (1996) found a weak positive correlation between egg diameter and emergence in rainbow trout and Koski (1975) also suggested the possibility of such a relationship in his research on chum salmon.

This evidence supports H2, that fish from small eggs may require exogenous sources of nutrition at an earlier date and thus are required to emerge from gravels early. Early emergence in nature has been correlated with poor survival due to increased mortality from depensatory predation and reduced prey abundance (Taylor 1980). Depensatory predation may occur on early emerging fry because the predator to prey ratio is high (Neave 1953). In addition, if fry hatch simultaneously then it is likely that a simultaneous emergence event would follow, thus chances for survival may be increased due to predator satiation. Increased mortality may also occur as a result of early emergence, because prey abundance and water temperatures are seasonally driven. Therefore early emerging fry may encounter periods of low prey availability and low water temperatures resulting in limited ability to locate and assimilate food (Bams 1969; Taylor 1980; Miller and Brannon 1982; Mortensen et al. 2000).

Yolk Weight

Yolk weight was significantly related to egg weight at all sampling episodes. Koski (1975), Kazakov (1981), Wallace and Aasjord (1984), and Beacham and Murray (1985) also found egg size positively related to yolk supplies. However, it is not particularly surprising that yolk weight was related to egg weight because unfertilized eggs consist almost entirely of yolk, therefore the majority of their weight is attributable to yolk. Likewise, at hatching, a substantial portion of a fish's total weight is yolk. Therefore, it is logical to assume that egg weight, or the amount of yolk that a fish starts with, will be related to the amount of yolk it possesses at a later date, especially during early development.

It is interesting to note however, that at Emergence, alevins derived from the largest eggs had more than two times the residual yolk of alevins derived from the smallest eggs. Burgner (1991) postulated that an extra supply of yolk might be beneficial when there are prey shortages or may provide additional time for incubation and growth. Kristjánsson and Vøllestad (1996) supported this claim when they found that rainbow trout from large eggs were able to survive longer when exogenous food was withheld. This is in contrast to Beacham et al. (1985) who found no relation between residual yolk and egg size.

Somatic Tissue Weight and Alevin Length

Egg weight effects on somatic tissue weight and alevin length were apparent throughout incubation but were especially prevalent at later development stages. These results are consistent with those found by Yastrebkov (1971), Koski (1975), Kazakov (1981), Springate and Bromage (1985), and Beacham and Murray (1990). Beacham et al. (1985) found no relationship between tissue weight and egg size. However, they observed only three females and two egg weight categories that were based on water hardened egg weight.

By Emergence, alevins derived from larger eggs were both heavier and longer. Alevins derived from eggs that weighed in the top 20% were more than 27% heavier and 7% longer than alevins derived from eggs that weighed in the bottom 20%. This size advantage would have likely been beneficial for survival in a natural environment due to advantages in foraging and predator avoidance. Large first-feeding fry would be more able to compete with other fry for food resources because they may be able to displace smaller fry and they are able to eat larger prey items because of their increased mouth gape. Balcer (1988), with rainbow smelt (*Osmerus mordax*), and Boubee and Ward (1997), with common smelt (*Retropinna retropinna* Richardson), reported prey size was correlated with fish length and mouth gape.

Swimming speed is also affected by body size or fish length. Arumugam and Geddes (1987) reported that the type and size of prey of first-feeding golden perch (*Macquaria ambigua* Richardson) fry was restricted by swimming speed. Increased swimming speed may also help large fry avoid predators (Healey 1982). Parker (1968, 1971) demonstrated that coho salmon preyed on pink salmon fry and had a strong bias for smaller individuals. Hiyama et al. (1972) also found increased mortality of small chum salmon fry in a small coastal stream.

Yolk Use Rate

Egg weight significantly affected instantaneous yolk use rate during four out of five intervals. During these intervals there was a tendency for alevins derived from smaller eggs to have proportionally greater yolk use rates. However, during the interval from Post-Hatch 3 to Emergence, egg weight did not significantly affect yolk use rate.

Perhaps the mixed results from this current investigation were caused by inconsistent growth and yolk use patterns. Growth of fish is frequently discontinuous (Weatherley and Rogers 1978). Specific growth rates should be estimated from longer periods of data; thus, it may be most accurate to use the data from Hatch to Emergence as the true yolk use rate and conclude that egg weight was indeed a significant determinant of yolk use rate.

Previous studies with rainbow trout are also inconclusive as to the relationship between yolk use and egg size. Pitman (1979) found that fry derived from large eggs had increased conversion efficiencies. Whereas, From and Rasmussen (1991) found energy conversion to be independent of egg size.

Somatic Tissue Growth Rate

Somatic tissue growth rates were significantly affected by egg weight during two out of five intervals analyzed. Similar studies have found mixed results relating egg size to growth rate. Gall (1974) and Pitman (1979) found higher growth rates among fry from larger eggs. Conversely, Wallace and Aasjord (1984) and Silverstein and Hershberger (1992) found fry derived from smaller eggs had higher growth rates. Further, Springate and Bromage (1985), From and Rasmussen (1991), and Kristjánsson and Vøllestad (1996) found no egg size effects on growth rate.

The intervals where egg weight significantly affected tissue growth were Post-Hatch 3 to Emergence and during the overall interval from Hatch to Emergence. The most extreme difference in growth rates between the egg weight groups was during the last interval before exogenous food would become available, Post-Hatch 3 to Emergence. Perhaps alevins from small eggs restricted their growth rate in order to conserve yolk or they may have been so deficient of yolk resources that they could not maintain their previous growth rates, unlike alevins derived from larger eggs. The positive relationship between somatic growth rate and egg weight is also borne out through the entire interval from Hatch to Emergence, suggesting that these results are not spurious relationships caused by discontinuous growth among various egg weights.

Fry

Survival

The regression of fry survival on egg weight was not significant. Previous research of egg size effects on fry survival has shown mixed results. Fowler (1972) and Pitman (1979) found decreased survival of fingerlings and fry from large eggs. In contrast, Jonasson (1993) found a positive correlation between egg size and survival and Wallace and Aasjord (1984) found increased "pin-head" mortality in fry from small eggs.

The relationship between fry survival and egg weight in this experiment may not be indicative of the relationship between fry survival and egg weight in nature for several reasons. First, the fry were fed rations in excess of true need, thus were not subjected to competition with other fry for food resources. When the fry were introduced into the growout tanks, there were significant weight and length differences between the egg weight groups. It is logical to assume that if food resources were in short supply, as might be the case in nature, larger individuals would most likely outcompete the smaller individuals and hence their survival may be enhanced. Second, predators were not present in the rearing environment. In natural environments, mortality due to predation can be substantial. Hunter (1959) estimated that up to 85% of Hooknose Creek fry are lost to predation in years when fry abundance is low. In addition, it has been shown that some predators are size-selective (Patten 1977; Parker 1971) and that larger fry are more able to avoid predators (Healey 1982). Thus the size advantage at emergence enjoyed by fry derived from larger eggs may have also led to increased survival due to their increased ability to avoid predators. Third, because there were differences in development time to hatch, there most likely would have been differences in emergence timing and, as discussed earlier, this may have impacted survival of the fry under natural conditions due to fluctuations in prey abundance and predation pressure. In this study, body size advantages and emergence time differences associated with the various egg weights were most likely negated by lack of competition and predation and thus differences in survival may have been mitigated.

Weight and Length

Egg weight had a significant effect on the weight and length of fry after 3 months of feeding. This result was consistent with other research that has identified significant positive relationships between egg size and subsequent fry length or weight (Yastrebkov 1971; Fowler 1972; Gall 1974; Wallace and Aasjord 1984; Beacham and Murray 1990; Silverstein and Hershberger 1992; Jonasson 1993). However, Springate and Bromage (1985) found no egg size effect on fry size after 4 weeks of exogenous feeding. In this study, there was a general trend of increasing fry size associated with larger egg weights. This result suggests that fish from relatively small eggs tend to remain relatively small well into fry development and as discussed above, reduced body size is associated with a lesser ability to forage and avoid predators.

The positive relationship between egg size and fry size in this experiment may actually be less pronounced than in nature because there was a surplus of feed available. In nature, there may, depending on food availability, be competition between large and small fry for food resources. If food resources are limiting, the larger fry would be favored due to their greater ability to utilize and compete for resources. Thus, the advantages imparted to larger fry may amplify differences in fry size as food supply decreases.

CHAPTER II.

QUANTITATIVE GENETIC ANALYSIS OF SURVIVAL OF PINK SALMON EMBRYOS

INTRODUCTION

In nature, the survival of salmon embryos is related to many factors. Eggs and embryos in stream gravel are subject to varying biotic and abiotic environmental factors that influence survival. Biotic mechanisms include spawner density which determines redd superimposition, fungal parasitism, and predation by birds, insects, and fish. Abiotic factors influencing embryo survival include water flow, water temperature, and water quality. Genetic variation of susceptibility to mortality factors also may influence embryo survival.

Because survival is closely related to fitness, theory suggests that population variability of survival should have little heritable genetic basis. According to Fisher's fundamental theorem, a population at evolutionary equilibrium would not be expected to possess additive genetic variation in total fitness as a result of natural selection (Fisher 1958). Additive genetic variance is a cause of resemblance between relatives (Falconer 1989). Differences of survival between families would imply that genetic variation of survivability is present in the population. An inherited difference in family-specific survival has implications for management because it indicates that families may contribute genetic information to subsequent generations in varying amounts. This variation in family-specific genetic contribution suggests that the effective population size, a genetic concept related to the amount of inbreeding in the population, is reduced (Falconer 1989). Therefore, populations managed for escapement may have reduced effective population numbers because a high percentage of spawners may be close relatives.

Geiger et al. (1997) demonstrated significant additive genetic variation was present in marine survival of pink salmon from saltwater entry to return to spawn. The present research attempts to determine if additive genetic variation exists in the survival of pink salmon during early freshwater incubation in a laboratory environment and is part of a larger study (Geiger et al. in prep.). In laboratory incubation, the biotic and abiotic environmental factors acting on embryonic survival in nature are mostly controlled; thus differences in survival due to environmental differences are reduced. This experiment compared the embryonic survival of paternal half-sib families -- each male was mated with two females. Hierarchical mating designs allow for the partitioning of variance into its underlying genetic and non-genetic components (Becker 1984). By comparing the

mean squares due to males with the mean squares due to females nested within males, *F* statistics were generated to determine whether male effects on survival were significant. Significant additive genetic variation was inferred when significant male effects were present.

MATERIALS AND METHODS

Overview – The survival of pink salmon embryos was studied in relation to quantitative genetic effects. A nested mating design allowed for the partitioning of variance into its underlying components. Effects due to sires and dams were estimated with an analysis of variance (ANOVA).

Gamete Acquisition and Culture

Study Population

Pink salmon gametes were obtained from the Gastineau Hatchery in Juneau, Alaska. Douglas Island Pink and Chum (DIPAC) operates Gastineau Hatchery and obtained their pink salmon broodstock from nearby Salmon Creek. Eggs were collected from a stratified, with respect to average egg diameter, sample of females. Fertilization and incubation occurred at Kowee Creek Hatchery on Douglas Island about 3 km from the Gastineau Hatchery and Salmon Creek.

Mating Design and Incubation

On 31 August 1996 gametes were collected and fertilization was completed. Semen from 30 spermiating males, selected at random, and eggs from 60 fully ovulating females were obtained. Semen, collected by expression, and eggs, obtained by excision, were placed in dry labeled containers. Sperm motility was verified by microscopic observation. Gametes were transported on ice to Kowee Creek Hatchery.

A nested hierarchical mating design was employed to create 60 half-sib families. Sperm from a single randomly chosen male fertilized eggs from two females randomly chosen from among all females. Each full-sib family (single male mated to single female) was split into two replicates and randomly placed into 120 compartments of FAL Heath™ incubator trays. Fertilization was by the dry method and fertilized eggs were rinsed before being placed into incubator compartments. The fertilized eggs were incubated in free flowing ambient Kowee Creek water.

Data Analysis

Observations

An initial pick-off of "blank" eggs was performed on 1 September 1996. On 18 October 1996, eyed eggs were physically shocked and mortalities counted and removed. Ambient temperatures and mortalities were recorded so that accumulated temperature units (ATU) and survival could be determined. Survival was calculated during the interval from initial pick-off to eyeing.

Lack of fertilization would result in the overestimation of embryo mortality. Therefore, at 16 hours post fertilization, 10 eggs from each full-sib cross (5 from each replicate) were preserved in a 10% formalin solution. These samples were used to verify the fertilization of the crosses. Preserved eggs were removed from solution, rinsed with fresh water, then fixed in 10% acetic acid for 2 minutes. Samples were then placed in alcohol and examined under a dissecting microscope for cell cleavage. Cleavage was observed in greater than 90% of all samples thus all crosses were retained in the experiment.

Statistical Model

In order to stabilize variance, survival data was transformed to its empirical logit (Agresti 1990)

$$\text{logit (survival)} = \log\left[(y_i + \tfrac{1}{2})/(n_i - y_i + \tfrac{1}{2})\right]$$

where y_i is the number of mortalities and n_i is the initial number alive of the i^{th} replicate incubator. Embryo survival was analyzed with an unbalanced, nested, random effects analysis of variance (ANOVA) model (Becker 1984; Searle 1971). The following statistical model partitioned the variance attributable to the main effects, male, and female within male.

$$Y_{ijk} = \mu + M_i + F_{j(i)} + e_{ijk}$$

where Y_{ijk} is the logit of survival of the k^{th} observation of the j^{th} female nested within the i^{th} male; μ is the population mean; M_i is the effect due to male (i = 1...30); $F_{j(i)}$ is the effect due to females (j = 1,2) nested within males; and e_{ijk} is random error. *F*-statistics were created by comparing mean squares sequentially as determined by the expected mean squares. For example, the mean square for male was divided by the mean square for female within male and the mean square for female within male was divided by the mean square error term.

One female had extremely poor survival, 10.1% averaged between replicate half-sib crosses. Presumably, this female's eggs were overripe. Her progeny were removed from the analysis and Type III sums of squares (Milliken and Johnson 1984) were used due to the resulting unbalanced data. Both males and females were considered random factors. Statistical analyses were performed with the GLM (Generalized Linear Model) procedure in the statistical program SAS, Release 6.12 (SAS Institute Inc. 1996).

RESULTS AND DISCUSSION

Overall survival to eyeing for all half-sib families ranged from 53% to 99% and averaged 92% ±0.86%. Analysis of variance determined that female within male effects on survival were significant ($P<0.0001$) (Table 1). A significant female effect indicates the presence of maternal genetic effects and or environmental effects held in common among eggs from the same female. Egg size and egg quality differences between individual females are possible causes for common environmental effects, but egg size did not significantly affect survival in this experiment (see Chapter 1). Therefore, egg quality may have contributed to the significant female effect. Regardless, maternal genetic and environmental effects, held in common among eggs from individual females, cannot be partitioned with this analysis.

Analysis of variance also indicated a significant male effect on survival ($P=0.043$) (Table 1). A significant male effect indicates the presence of additive genetic variation. Withler et al. (1987) reported similar results finding significant male effects on survival to the eyed stage of chinook salmon (*Oncorhynchus tshawytscha*). However, Beacham and Murray (1987) did not find a significant male effect on embryonic survival of pink salmon but their research lacked statistical power because only 5 males were used.

Other researchers have reported on early survival of salmonids with respect to quantitative genetics. However, most report heritability estimates, the ratio of additive genetic variance to total phenotypic variance (Falconer 1989), without reporting the probability significance of the additive genetic variation. In the present study, heritability of survival, based on the male component of variance, was estimated to be 1.15 with a standard error of 0.69 (Table 2). However, most researchers report lower heritabilities for survival of salmonids in laboratory culture. Robison et al. (1984) estimated the heritability of survival to eye in brook trout (*Salvelinus fontinalis*) was 0.09 ±0.05 and Rye et al. (1990) estimated a 0.08 ±0.02 heritability of survival from the eyed stage to hatching for both Atlantic salmon and rainbow trout. Gall and Gross (1978) found heritability of survival to the eyed stage of three stocks of rainbow trout to be 0.09 ±0.11, 0.19 ±0.11, and 0.40 ±0.13. Other research has found less definitive evidence supporting genetic influences on early survival. Kanis et al. (1976) estimated heritabilities for survival to the eyed stage of 0.12 ±0.03, 0.01 ±0.03, and -0.10 ±0.08 for Atlantic salmon (*Salmo salar*),

brown trout (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*), respectively. Ayles (1974) estimated the heritability of survival to the eyed staged of splake hybrids (*Salvelinus fontinalis x S. namayacush*) to be 0.06 ±0.07 and Withler et al. (1987) estimated the heritability of survival to eyeing among six stocks of chinook salmon to be 0.03 ±0.07.

The fact that many researchers have found low heritabilities of survival for salmonids supports Fisher's fundamental theorem, which implies that traits closely associated with fitness should have little additive genetic variation. Mousseau and Roff (1987) reported heritabilities of what they termed "life history" traits for 75 species of invertebrates, ectothermic vertebrates, and endothermic vertebrates were significantly lower than heritabilities of morphological traits. However, because the life history trait heritabilities averaged 0.26, they contend, contrary to Fisher's fundamental theorem, that additive genetic variation of life history traits is still maintained in natural populations. Price and Schluter (1991) suggest that life history traits are affected by additional random environmental factors because they are one step further down the causal pathway from genes to phenotype and hence have low heritabilities.

An additive genetic component of variation associated with fitness or survival might seem to imply that each generation becomes more fit than the previous generation. However, because natural selection increases the fitness of individuals for conditions in the immediate past, the fitness of the same individuals in the present or future may be different because of changes in the environment (Price 1972). McIntyre et al. (1988) demonstrated inherited family-specific differences in survival from smolt to adult of coho salmon (*Oncorhynchus kisutch*) but descendants of the best surviving families in the first two generations actually had poorer survival in subsequent generations. These results suggest that changes in the environment tend to maintain additive genetic variation for fitness traits including survival.

Variation in the environment may differentially favor families at different times. In periods of poor overall survival, families with high relative survival most likely donate substantially to the gene pool of subsequent generations. This disproportionate gene donation reduces the effective population size because it increases the variance of family size (Falconer 1989). In populations managed for minimum escapement levels and in populations threatened by extinction it is important to realize that, because

survival differs among families, the number of spawners may not be a good estimate of the effective population size. In addition, spawning adults vary widely in their ability to reproduce successfully. Females have wide ranges in fecundity and males vary in their ability to compete for females. These differences between individuals in reproductive success may also contribute to a reduction in effective population size, although Geiger et al. (in prep.) make the argument that differences in family-specific survival, particularly during marine life, influence effective population size on a much greater scale. In order to maintain the health of pink salmon populations, increased care must be taken to maintain genetic diversity in light of the differences in family-specific survival and environmental fluctuations.

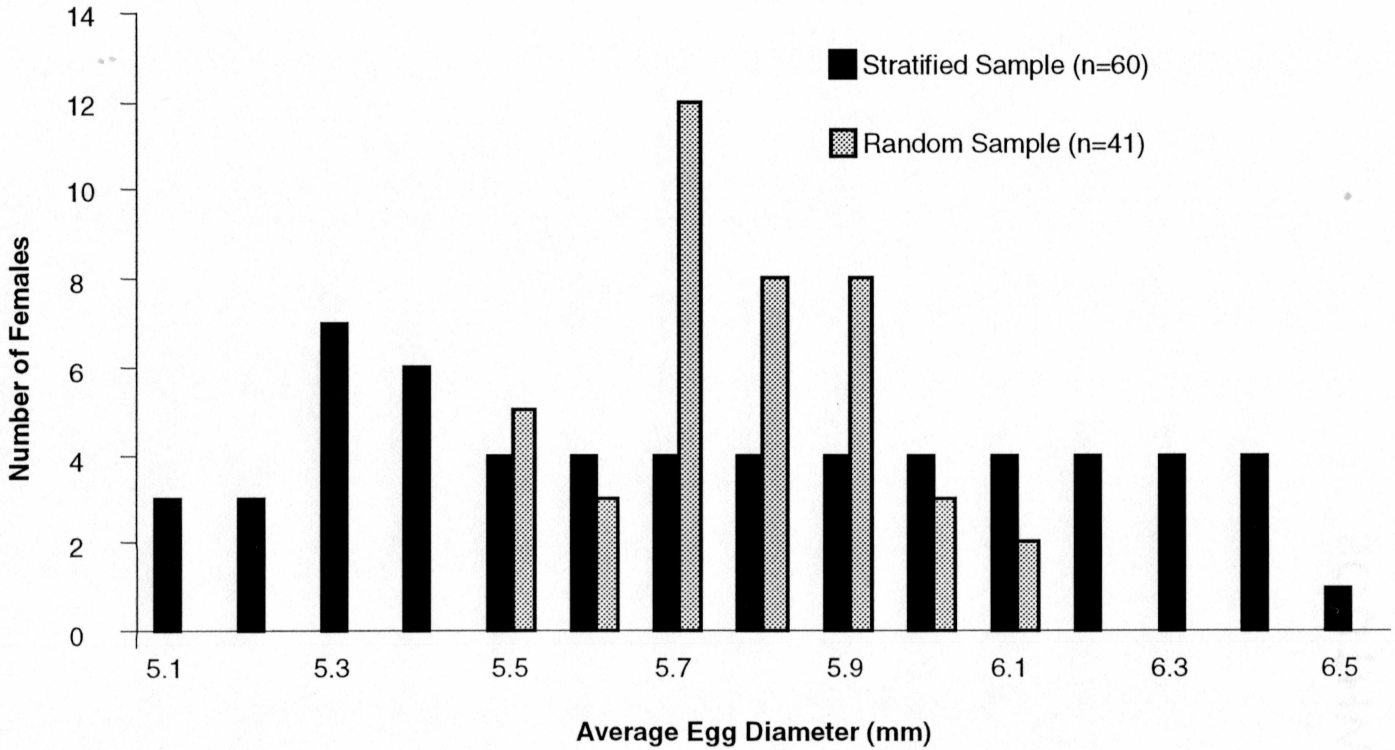


Figure 1. Frequency distributions of females in random and stratified (with respect to egg diameter) samples of Gastineau Hatchery pink salmon. Egg diameter is an average of ten unfertilized eggs from fully ovulating females.

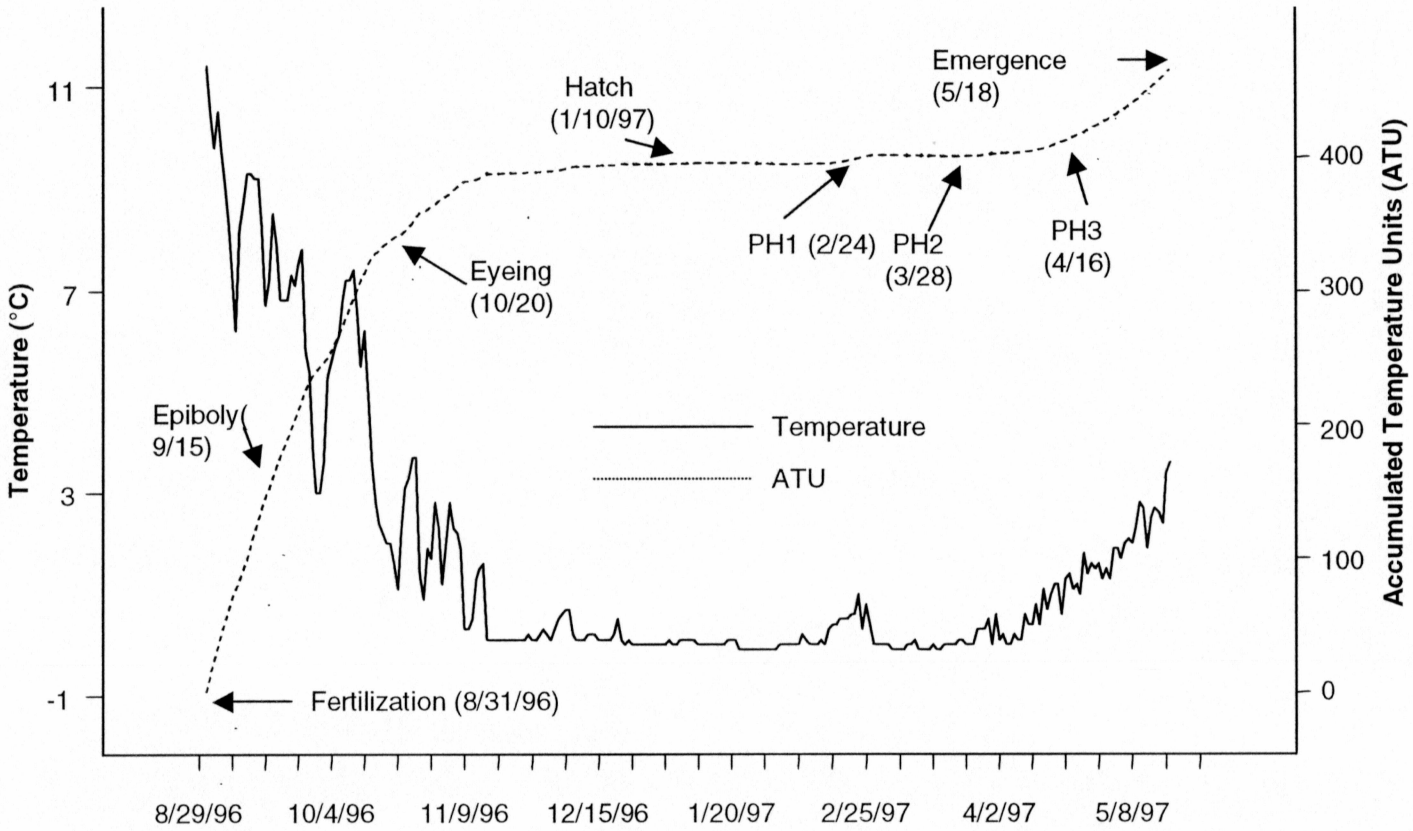


Figure 2. Sampling schedule and profiles of daily temperatures and accumulated temperature units (ATU) of incubating pink salmon. Samples were collected on eight dates; 31 August 1996 (Fertilization), 15 September 1996 (Epiboly), 20 October 1996 (Eyeing), 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

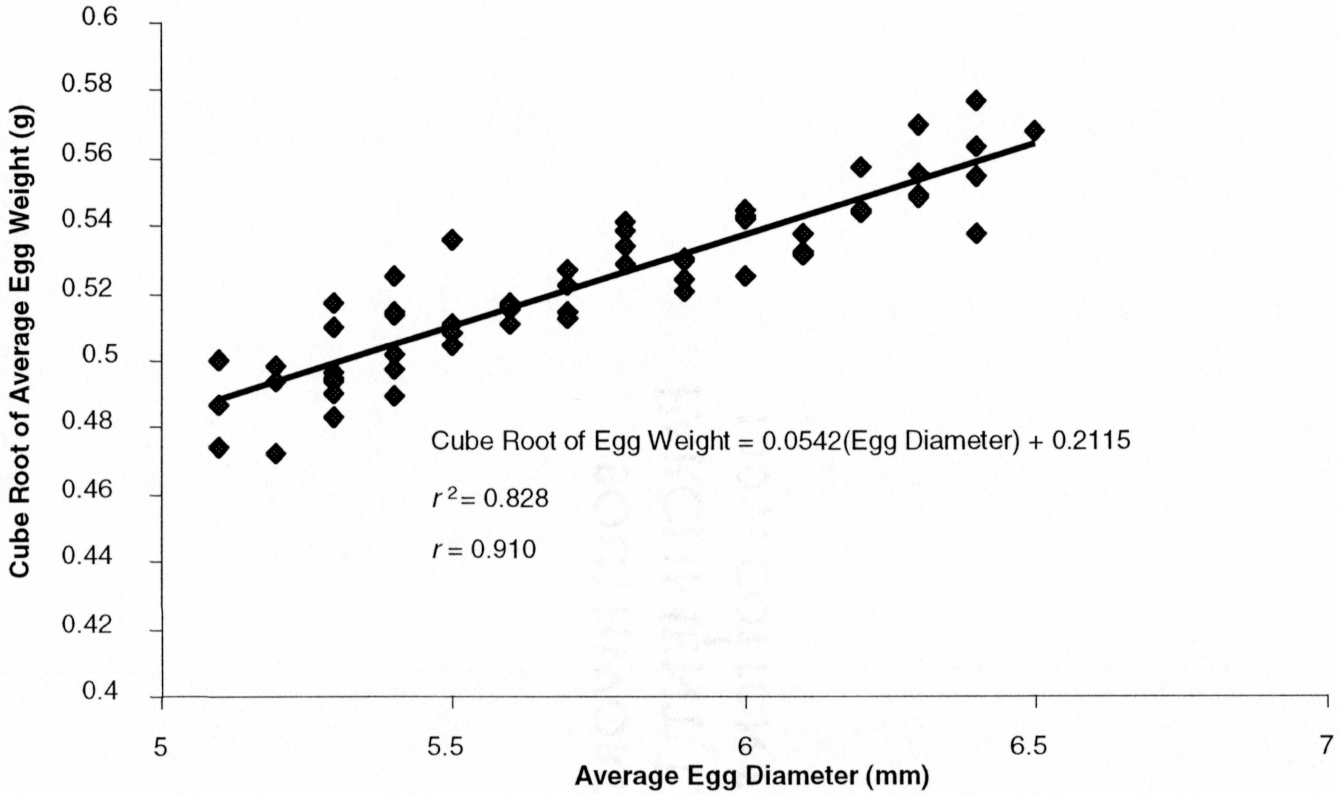


Figure 3. Linear regression of the cube root of average egg weight on average egg diameter. Egg diameter is an average of ten unfertilized eggs from a single female. Egg weight is an average of at least 30 unfertilized eggs from a single female, blotted dry.

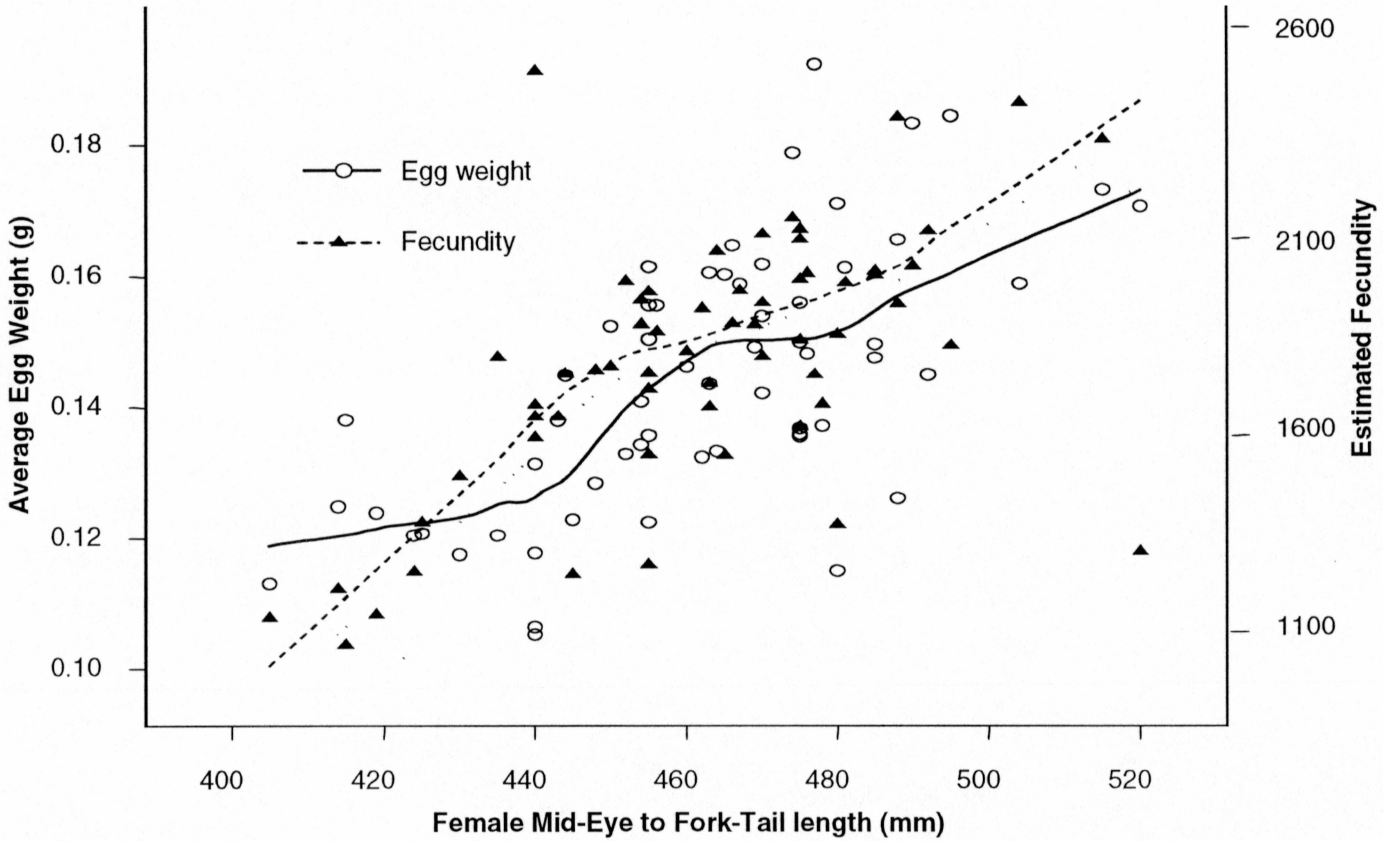


Figure 4. Local regressions of egg weight and fecundity on adult female pink salmon length. Local regression performed with the Loess function in S-Plus 4.5 using a span of 0.75. Egg weight is an average of at least 30 unfertilized eggs from a single female, blotted dry. Fecundity was estimated by multiplying egg mass total weight by the number of eggs per unit of weight in a weighed subsample of eggs from a single female.

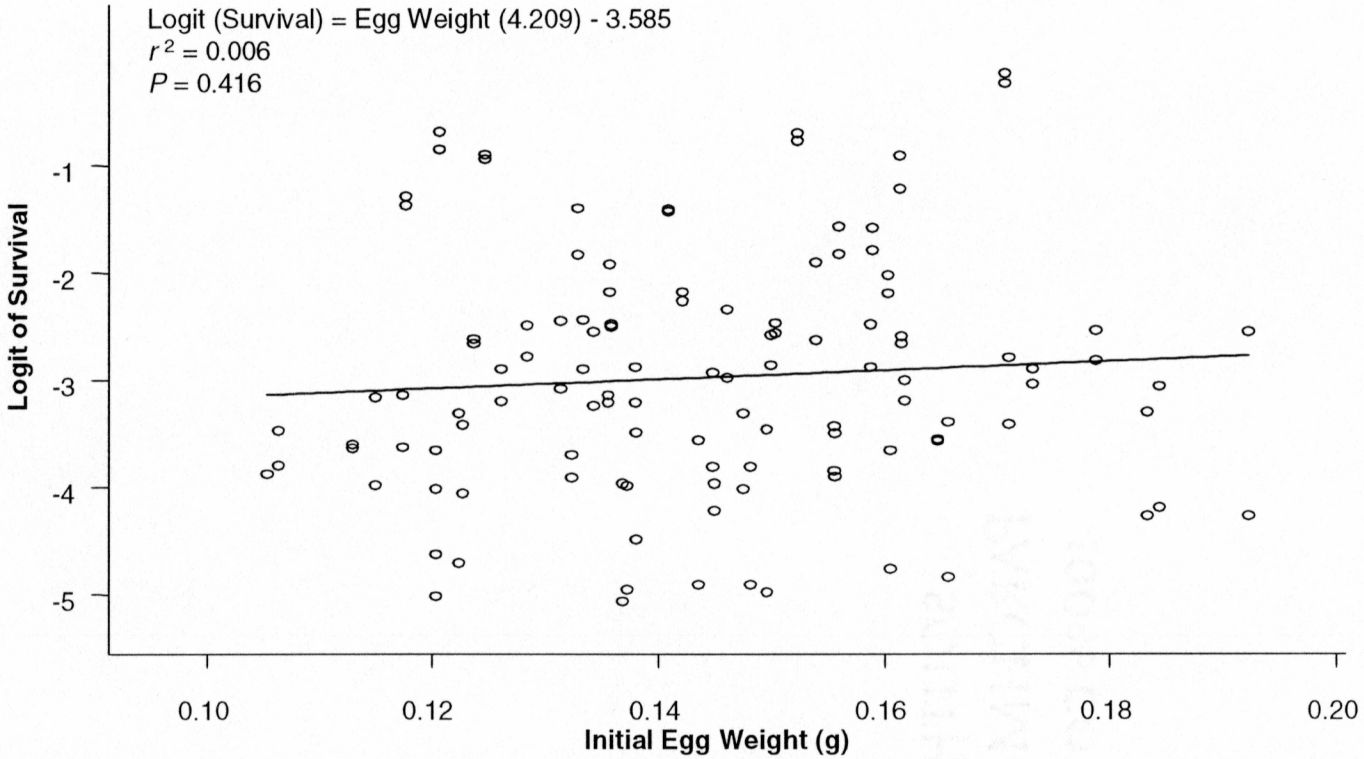


Figure 5. Linear regression of pink salmon survival to eyeing on initial egg weight. Survival data was transformed to the empirical logit (see text for explanation).

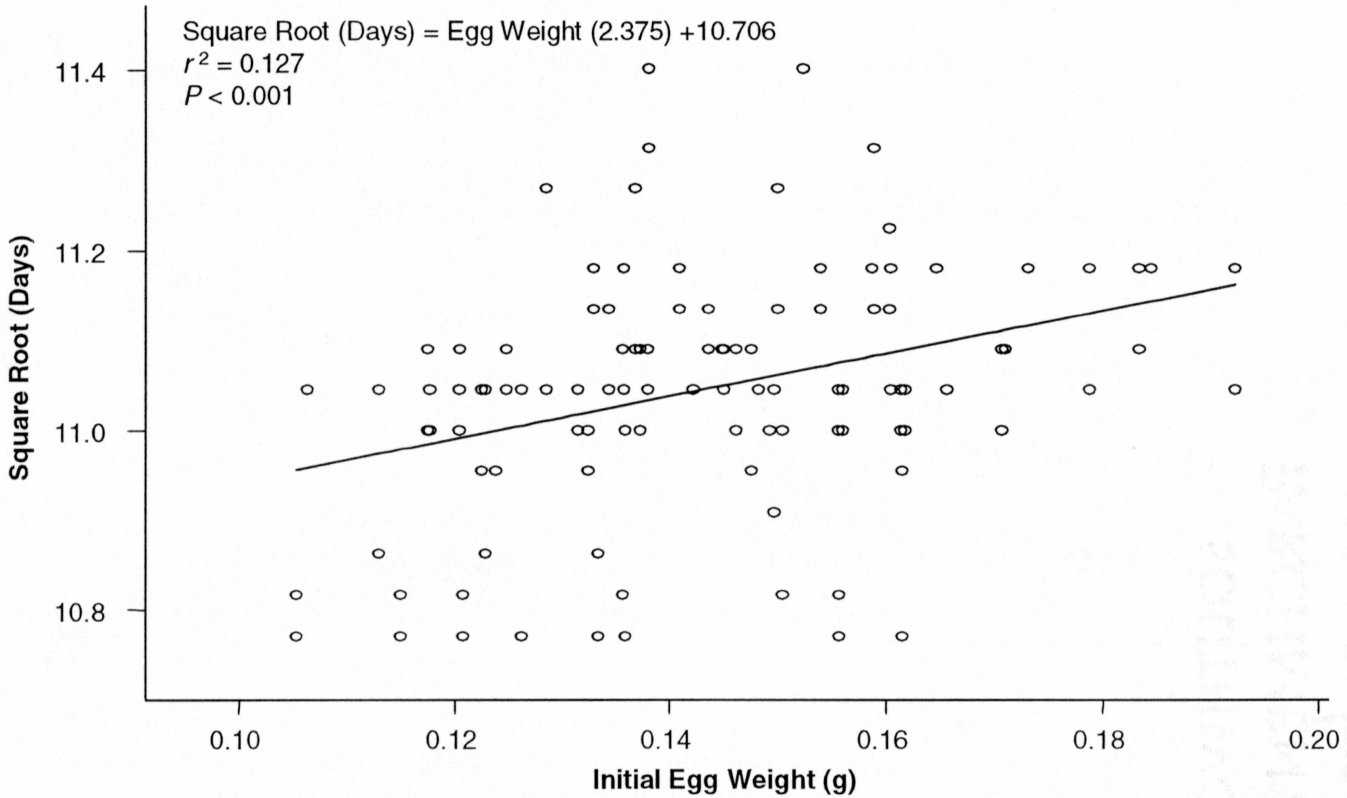


Figure 6. Linear regression of pink salmon development time to hatch on initial egg weight. Development time, in days, was transformed with the square root function.

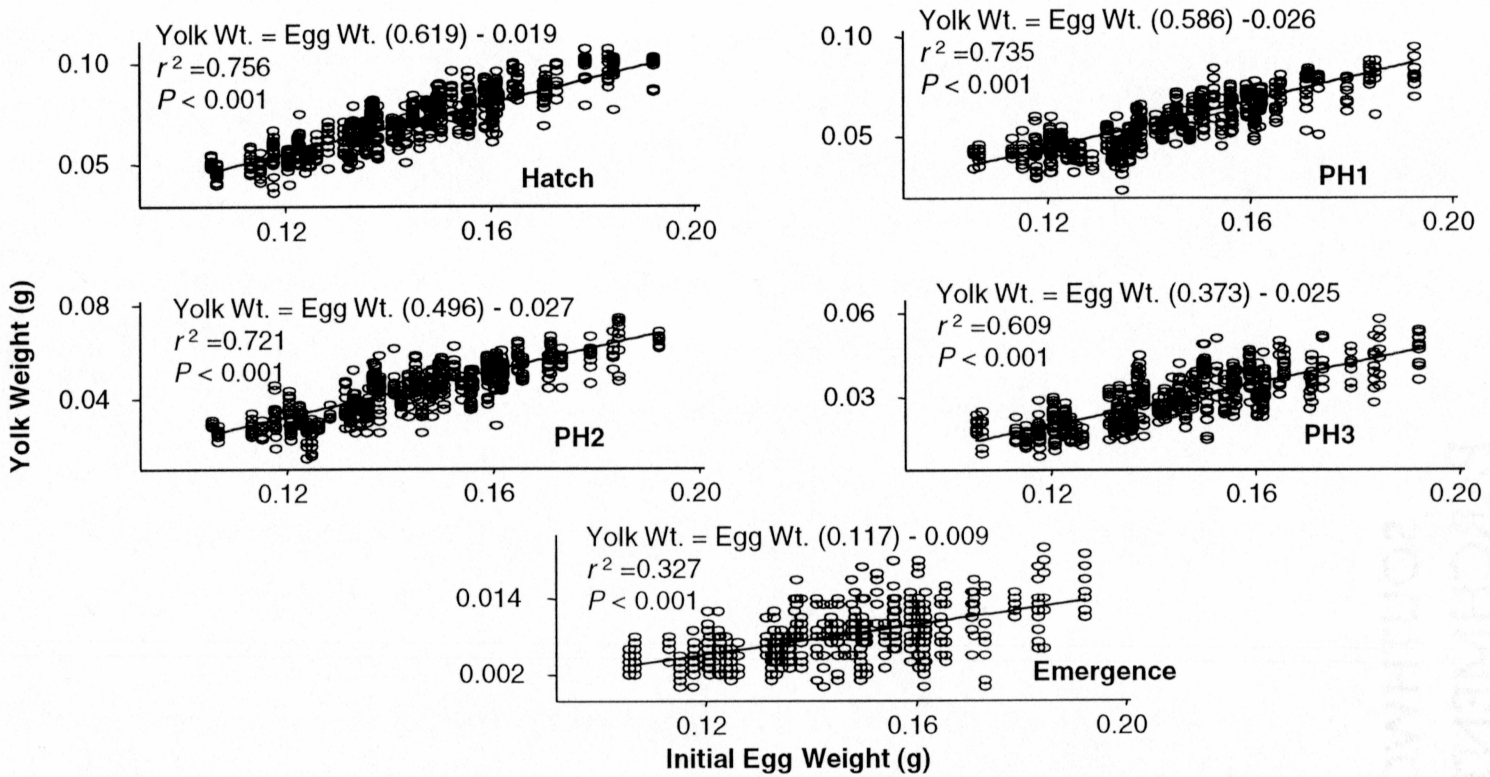


Figure 7. Linear regression of yolk weight on initial egg weight of pink salmon alevins at five sampling episodes. Sampling episodes occurred on 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

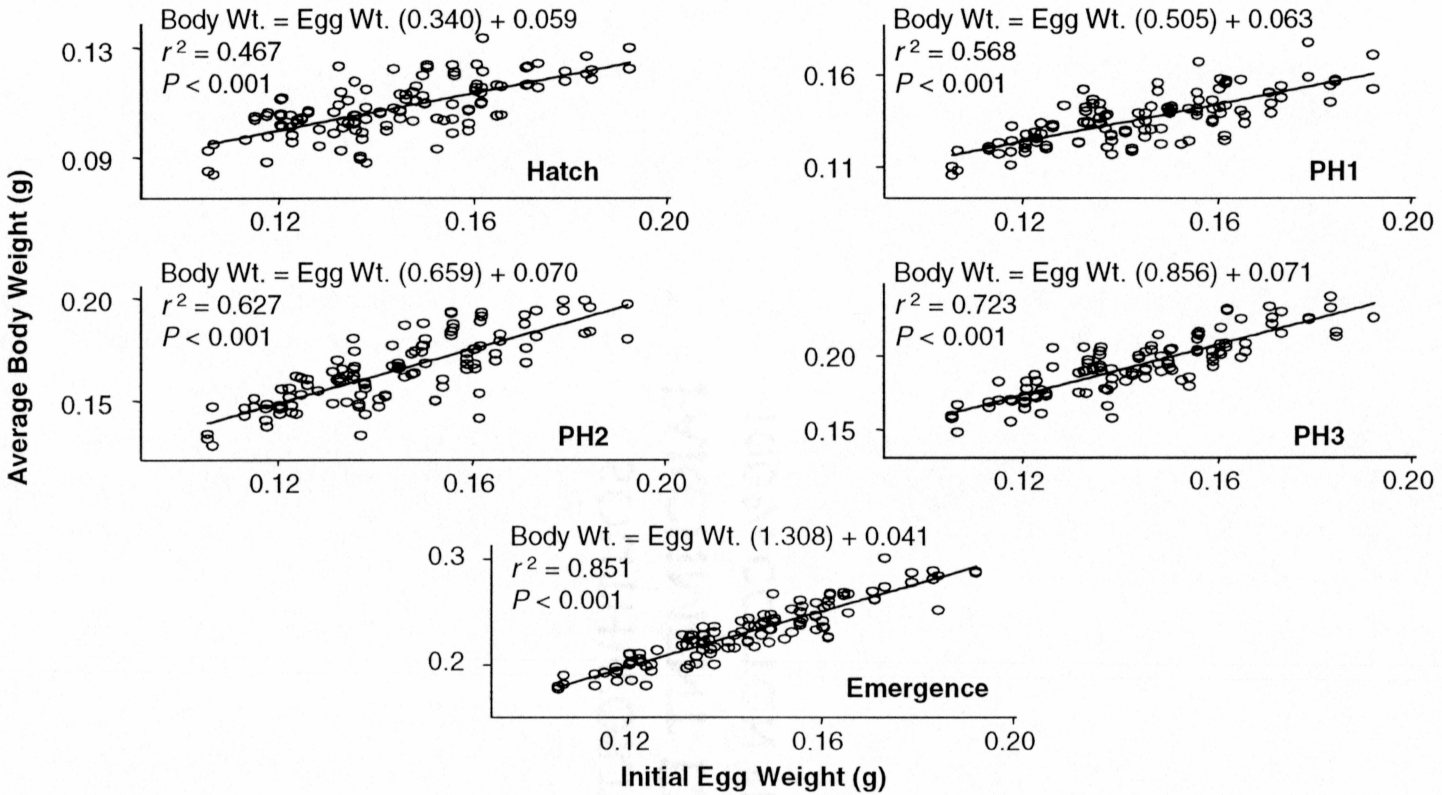


Figure 8. Linear regression of average somatic body weight on initial egg weight of pink salmon alevins. Somatic tissue of alevins was weighed after the removal of yolk mass. Tissue weights are averages of five alevins per replicate female. Sampling episodes occurred on 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

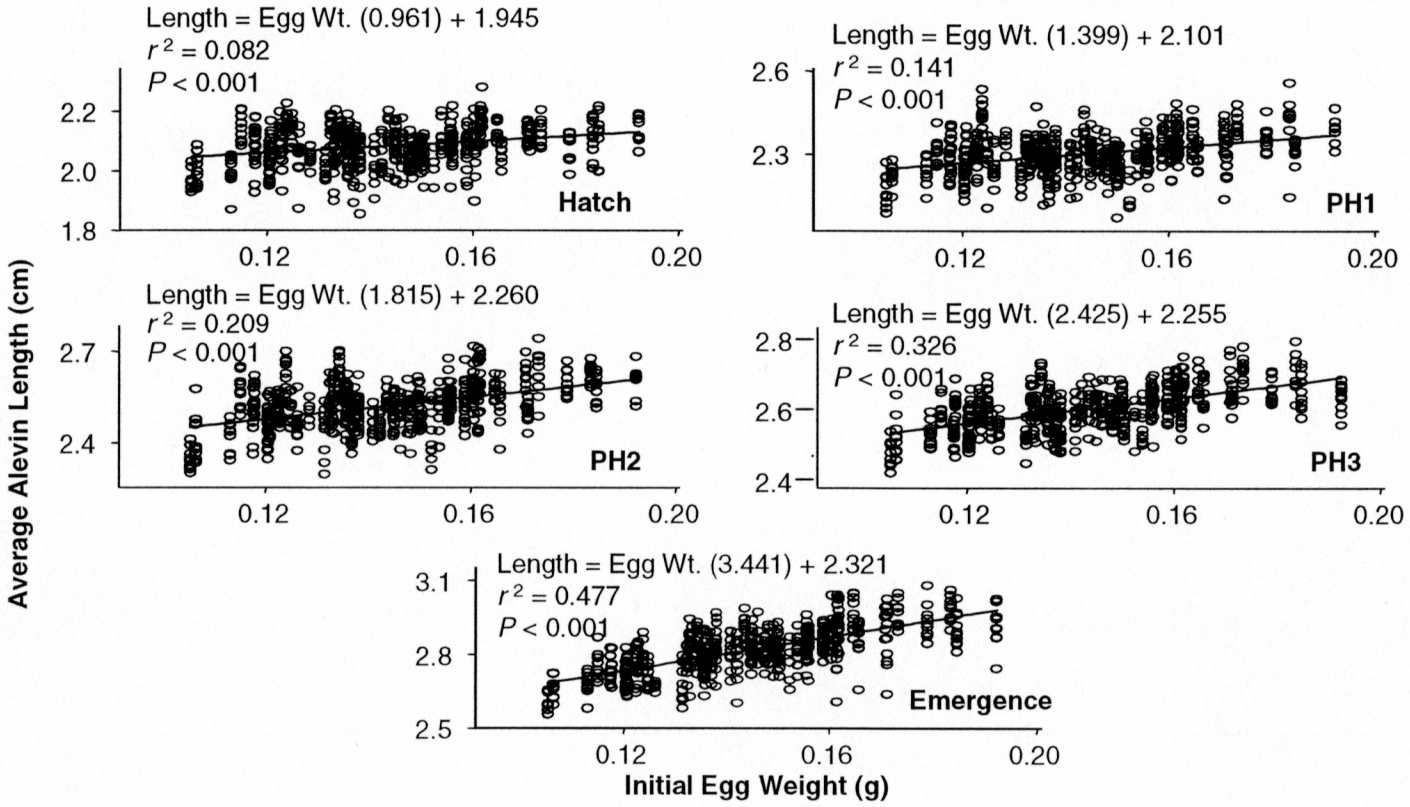


Figure 9. Linear regression of alevin length on initial egg weight of pink salmon at five sampling episodes. Alevin length was measured from mid-eye to the termination of the caudal peduncle. Sampling episodes occurred on 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

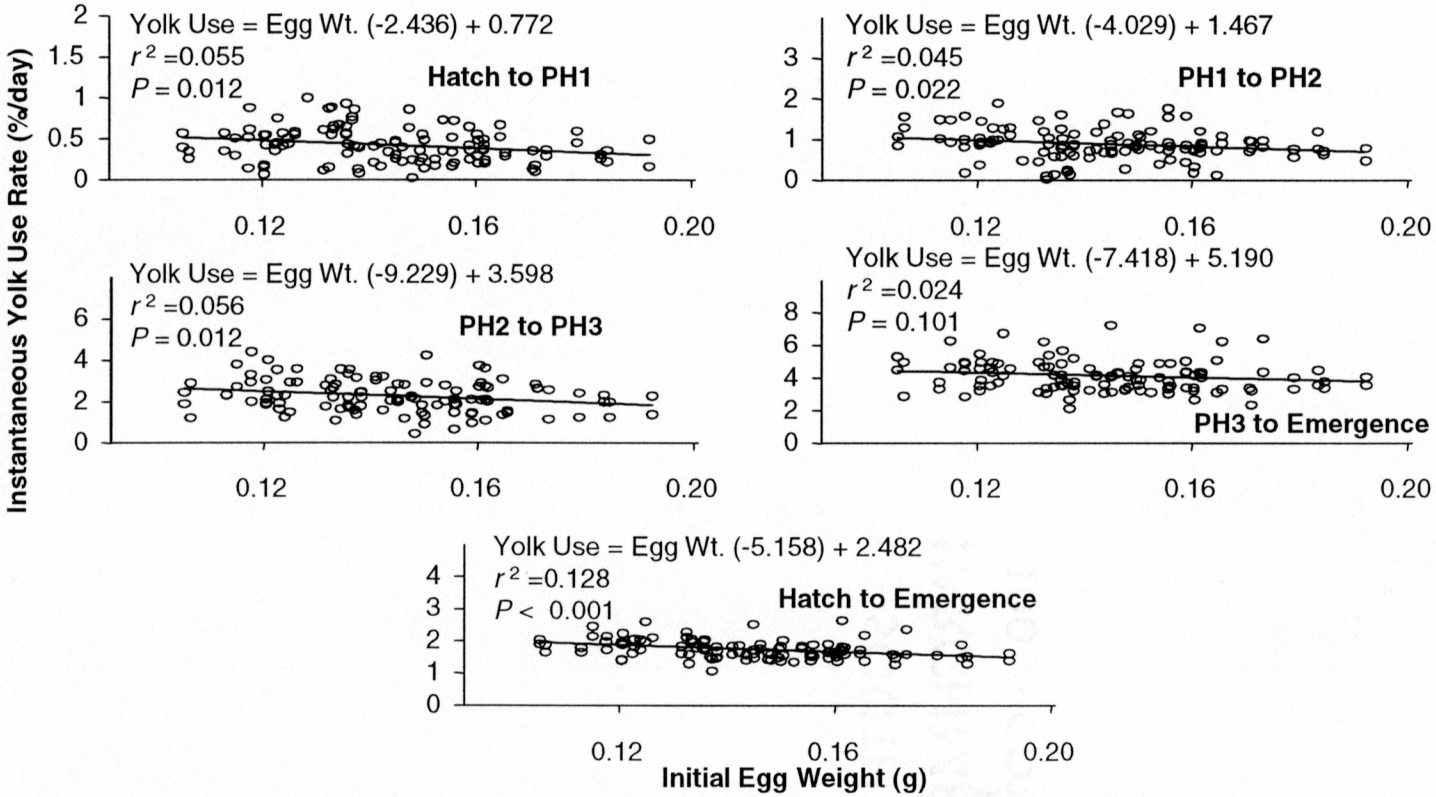


Figure 10. Linear regression of instantaneous yolk use rate on initial egg weight of pink salmon alevins during five intervals. Yolk use rates were calculated as instantaneous rates. Sampling episodes occurred on 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

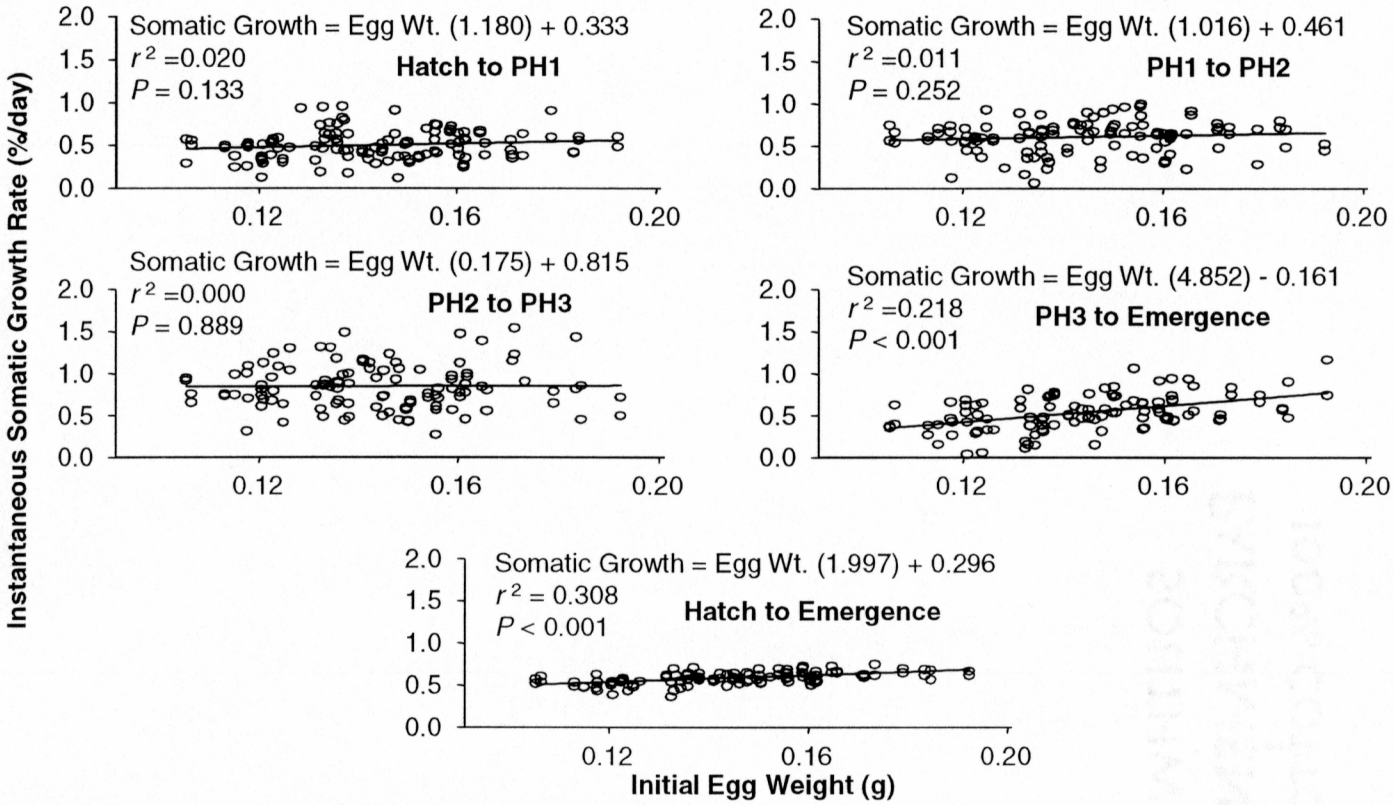


Figure 11. Linear regression of instantaneous somatic growth on initial egg weight of pink salmon alevins during five intervals. Somatic tissue was measured after the removal of yolk mass and somatic tissue growth rate was calculated as an instantaneous rate. Sampling episodes occurred on 10 January 1997 (Hatch), 24 February 1997 (Post-Hatch 1 (PH1)), 28 March 1997 (Post-Hatch 2 (PH2)), 16 April 1997 (Post-Hatch 3 (PH3)), and 18 May 1997 (Emergence).

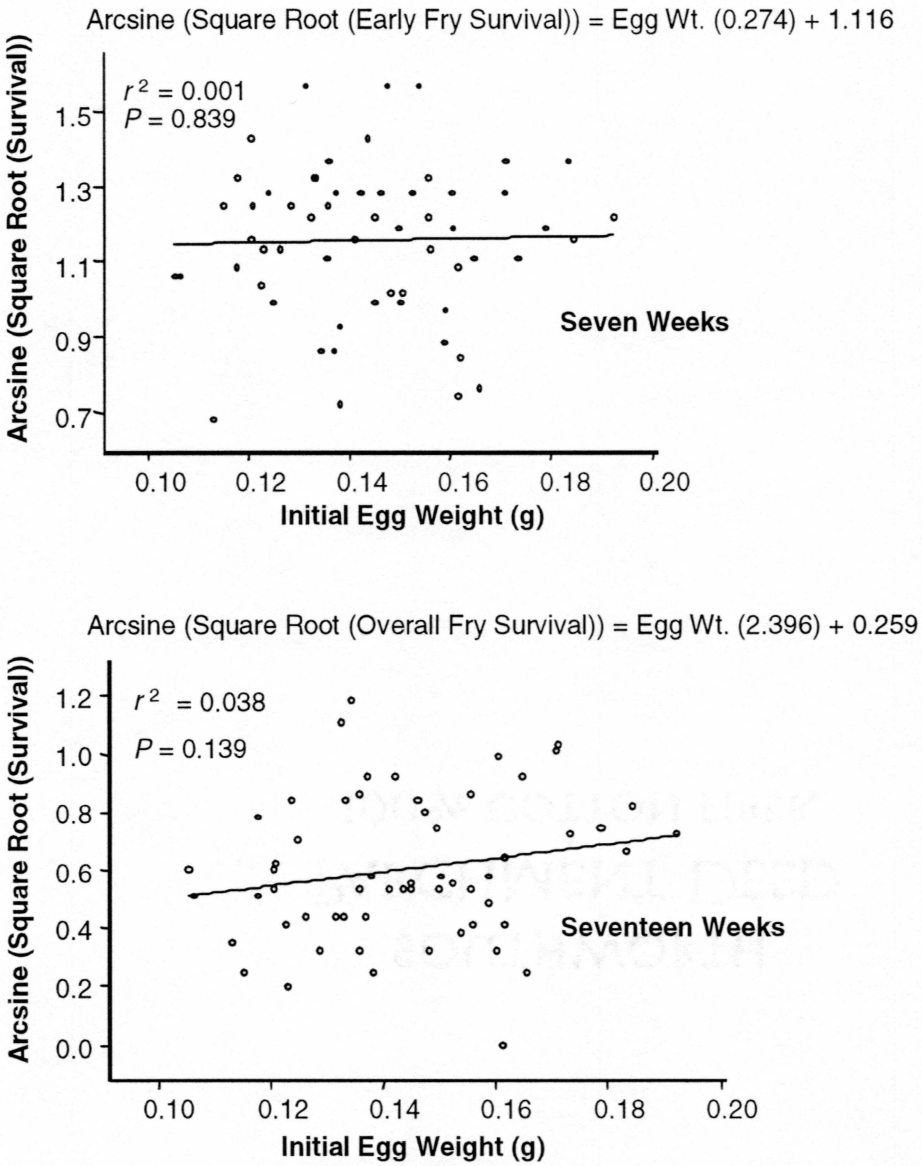


Figure 12. Linear regression of fry survival on initial egg weight of pink salmon. Survival was measured at 7 weeks (12 April 1997 to 3 June 1997) and 17 weeks (12 April 1997 to 8 August 1997) post-ponding. Fry survival was based on the survival of 50 fry from each of 59 females. Survival data was transformed with the arcsine square root transformation.

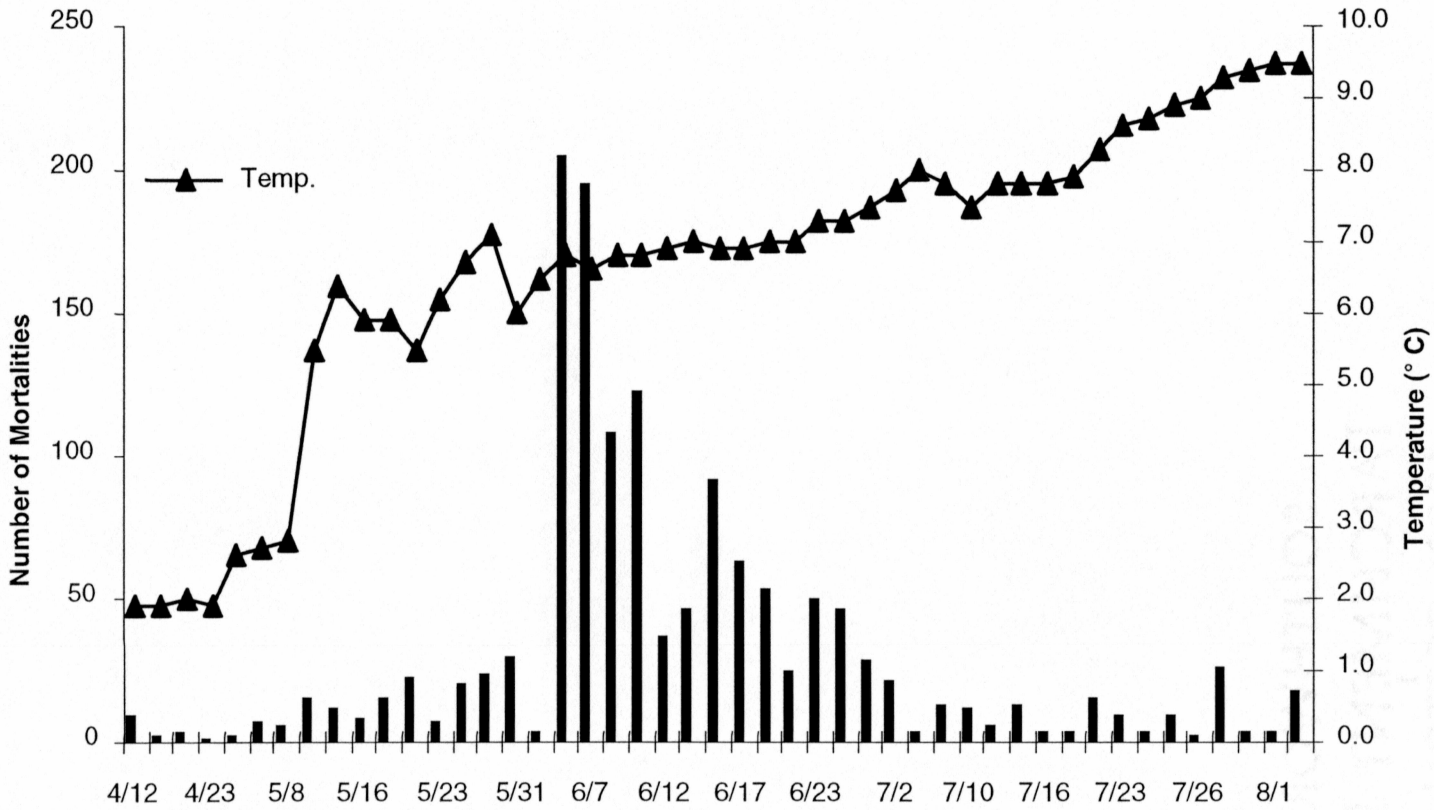
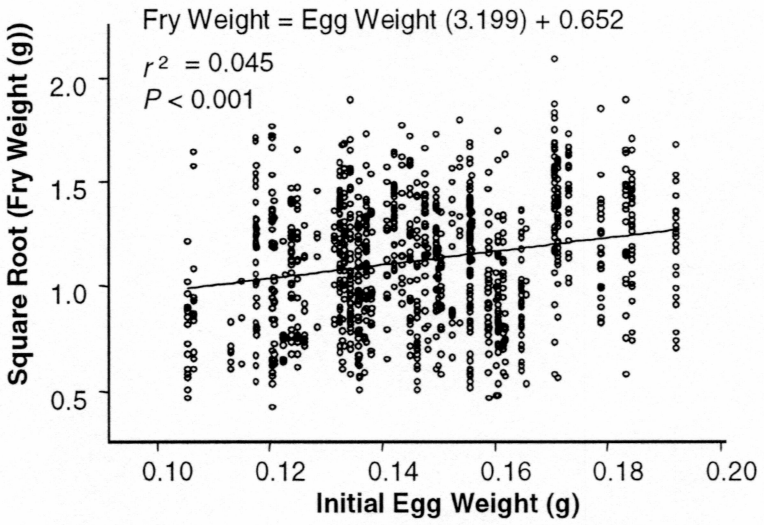


Figure 13. Frequency distribution of pink salmon fry mortality and temperature profile with respect to date. Number of mortalities is the total number of mortalities for all tanks on a given day.



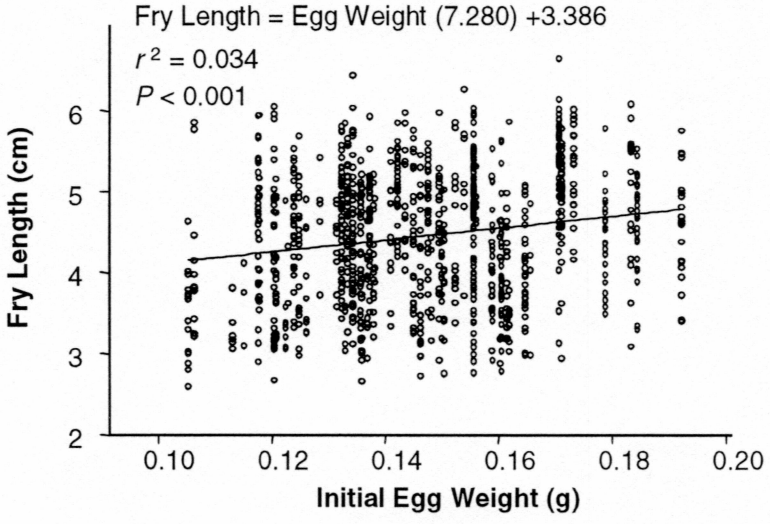


Figure 14. Linear regressions of fry weight and length on initial egg weight of pink salmon after 17 weeks of exogenous feed. Length and weight data was obtained from the surviving fry from each of 59 females on August 8 1997. Fry weight data was transformed with the square root function.

TABLE 1. Analysis of variance of quantitative genetic effects on pink salmon survival to the eyed stage. *F* statistics and significance levels were calculated with type III sums of squares that were generated with SAS release 6.12 by the model $Y_{ijk} = \mu + M_i + F_{j(i)} + e_{ijk,}$ where Y_{ijk} is the logit of survival of the k^{th} replicate incubator of the j^{th} female nested within the i^{th} male; μ is the overall population mean; M_i is the effect due to males (i = 1...30); $F_{j(i)}$ is the effect due to females (j = 1,2) nested within males; and e_{ijk} is random error.

	Male Effect				Female within Male Effect			
Trait	*F* Stat	dfM[a]	dfF[a]	*P* value	*F* Stat	dfF[a]	dfE[a]	*P* value
Survival	1.916	29	29	0.043	6.603	29	59	4.99E-10

[a] dfM, dfF, dfE = degrees of freedom for males, females nested within males, and error

TABLE 2. Quantitative genetic variance components and heritability estimate of pink salmon survival to the eyed stage. s^2male is variance due to male; s^2female is variance due to female nested within male; s^2phen is total phenotypic variance; h^2 is heritability based on the male components of variance; and $SE(h^2)$ is the standard error of the heritability estimate.

	s^2male	s^2female	s^2phen	h^2	$SE(h^2)$
Survival to Eye	0.364	0.662	1.263	1.152	0.691

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APPENDIX I. Outlier treatment and data transformation.

Trait	Total Observations	Outliers Removed	Reason for Removal	Transformation
Alevin Survival	120	2	Overripe female	Logit
Time to Hatch	120	0	NA	Square Root
Yolk Weight				
Hatch	589	0	NA	NA
PH1	581	0	NA	NA
PH2	585	0	NA	NA
PH3	565	1	Extreme value	NA
Emergence	570	7	Bloated individuals	NA
Body Weight				
Hatch	118	0	NA	NA
PH1	117	1	Stunted	NA
PH2	117	0	NA	NA
PH3	113	3	Bloated, all were full sibs	NA
Emergence	113	0	NA	NA
Alevin Length				
Hatch	588	6	Bloated, all were full sibs	NA
PH1	581	5	Bloated, 4 of 5 were full sibs	NA
PH2	583	2	Extreme values	NA
PH3	565	0	NA	NA
Emergence	565	0	NA	NA
Yolk Use Rate				
Hatch to PH1	117	2	Nonsense value and extreme	NA
PH1 to PH2	117	1	Extreme leverage point	NA
PH2 to PH3	113	0	NA	NA
PH3 to Emergence	112	0	NA	NA
Hatch to Emergence	112	0	NA	NA
Growth Rate				
Hatch to PH1	117	0	NA	NA
PH1 to PH2	117	0	NA	NA
PH2 to PH3	113	1	Nonsense value	NA
PH3 to Emergence	112	0	NA	NA
Hatch to Emergence	112	0	NA	Data Squared

Trait	Total Observations	Outliers Removed	Reason for Removal	Transformation
Fry Weight	1038	0	NA	Square Root
Fry Length	1038	0	NA	NA
Early Fry Survival	59	0	NA	Arcsine(Square Root)
Overall Fry Survival	59	0	NA	Arcsine(Square Root)

APPENDIX II. Yolk Area analysis at Hatch, Post-Hatch 1, Post-Hatch 2, and Post-Hatch 3 using Optimas™ image analysis software.

Image analysis of yolk area may be a more desirable method than standard yolk weight measurements for determining yolk mass data. Image analysis is a rapid process and allows the preservation of intact specimens because dissection is not required. Therefore, verifying the feasibility and accuracy of such measurements would prove beneficial.

Measurements of yolk area at Hatch, Post-Hatch 1, Post-Hatch 2, and Post-Hatch 3 were taken with the Optimas™ image analysis system. Yolk area measurements were consistent with yolk weight measurements. Correlation coefficients between yolk weight and yolk area were r=0.71, 0.74, 0.72, and 0.62 at Hatch, Post-Hatch 1, Post-Hatch 2, and Post-Hatch 3, respectively. However, there was more variability in yolk area measurements compared to yolk weight measurements at later sampling episodes. Egg weight effects on yolk area were significant (P<0.0001) at all sampling episodes and similar to their effects on yolk weight.

Yolk area measurements at Emergence were not feasible because skin pigmentation and closure of the abdominal suture made it virtually impossible to obtain consistent measurements of the yolk mass. However, measurements taken at Hatch, Post-Hatch 1, and Post-Hatch 2 were consistent with yolk weight measures. The results of these observations support the conclusion that yolk area measurements are suitable for alevins that still possess a large quantity of yolk and are not fully pigmented. However, Yolk area measurements at later development stages are not suggested due to increased variability compared with yolk weight measurements.

APPENDIX III. Comparison of wet and dry weight of somatic tissue and yolk mass at fertilization and Post-Hatch 3.

Throughout this research, wet weight measurement of yolk, tissue, and body mass were observed. In order to determine the validity of wet weight, as opposed to dry weight measurements, comparisons of wet weights with dry weights were performed on the fertilization and Post-Hatch 3 samples. Yolk mass and somatic tissue was weighed before and after 24 hours in a 60° C desiccating oven. Correlations between the dry weights and wet weights confirmed that wet weight was comparable with dry weight and that wet weight observations were an adequate measure of both yolk and tissue weight.

	Correlation coefficient (r) between wet and dry weight	
Sampling Episode	Yolk Weight	Tissue Weight
Fertilization	0.994	NA
Post-Hatch 3	0.968	0.934